

# Altered [ $^{125}$ I]Epidermal Growth Factor Binding and Receptor Distribution in Psoriasis\*

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Stimulation of growth and differentiation of human epidermis by epidermal growth factor (EGF) is mediated by its binding to specific receptors. Whether EGF receptors primarily mediate cell division or differentiation in hyperproliferative disease such as psoriasis vulgaris is unclear. To study the pathogenesis of psoriasis, 4-mm<sup>2</sup> punch biopsy specimens of normal, uninvolved, and involved psoriatic skin were assayed for EGF receptors by autoradiographic, immunohistochemical, and biochemical methods. Using autoradiographic and immunohistochemical methods, basal keratinocytes were found to contain the greatest number of EGF binding sites and immunoreactive receptors as compared to the upper layers of the epidermis in both normal epidermis and psoriatic skin. No EGF receptor differences between normal and psoriatic epidermis were

observed in this layer. In the upper layers of the epidermis, a 2-fold increase in EGF binding capacity was observed in psoriatic skin as compared with normal thin or thick skin. Biochemical methods indicated that [ $^{125}$ I]EGF binding was increased in psoriatic epidermis as compared with similar thickness normal epidermis when measured on a protein basis. Epidermal growth factor was shown to increase phosphorylation of the EGF receptor in skin. EGF receptors retained in the nonmitotic stratum spinosum and parakeratotic stratum corneum may reflect the incomplete, abnormal differentiation that occurs in active psoriatic lesions. Alternatively, retained EGF receptors may play a direct role in inhibiting cellular differentiation in the suprabasal layers. *J Invest Dermatol* 86:260-265, 1986

In normal human epidermis, epidermal growth factor (EGF) receptors, as detected by autoradiography following [ $^{125}$ I]EGF binding or by immunohistochemistry utilizing anti-EGF receptor antibody, were located primarily on the mitotically active basal keratinocytes and diminished in number as the cells became more differentiated [1-4]. A similar distribution of EGF binding sites was found in vitro [5], indicating that this distribution is not controlled by the dermis. To study the role of EGF receptors in differentiation, we selected a benign human skin disease, psoriasis vulgaris, because the affected keratinocytes may have both an abnormally increased proliferative capacity and an abnormally differentiated state. In active psoriatic skin lesions, as in normal skin, the actively dividing keratinocytes are limited to

the first 2-3 cell rows in the stratum basalis [6-9], the epidermal cell layer closest to the basal lamina. If EGF receptors were simply a marker of mitotically active cells, then the mitotically active basal cell layer in active psoriatic lesions should be the major site of increased numbers of EGF receptors. Conversely, if EGF receptors varied as a function of epidermal differentiation, an increased number of EGF receptors should be noted throughout the abnormally differentiating psoriatic epidermis. Previous studies had shown that large numbers of EGF receptors were present on cells in human skin that are metabolically very active but mitotically inactive such as sweat duct epithelium [2]. Epidermal growth factor receptors are also found on mitotically inactive arrector pili muscle and smooth muscle sites throughout the body [10]. Therefore, the correlation of EGF receptors with rapid cellular proliferation is imperfect [11]. The present studies indicate that a consistent increase in the number of EGF receptors is present in psoriatic lesions. However, the increased number of EGF receptors in active lesions was due to a persistence of EGF receptors primarily in sites of abnormal differentiation and not cellular division. Therefore, abnormal EGF receptor metabolism may play a role in the pathogenesis of the benign human hyperproliferative skin disease, psoriasis vulgaris.

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## Abbreviations:

- BSA: bovine serum albumin
- EGF: epidermal growth factor
- HBSS: Hanks' balanced salt solution
- HEPES: 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid
- HRP: horseradish peroxidase
- PBS: phosphate-buffered saline
- PDGF: platelet-derived growth factor

## MATERIALS AND METHODS

**Reagents** Epidermal growth factor was purchased from Collaborative Research (Waltham, Massachusetts), and [ $^{125}$ I]- and [ $^{32}$ P]ATP were obtained from New England Nuclear (Boston, Massachusetts). NTB-2 autoradiography emulsion was obtained from Eastman Kodak (Rochester, New York). Goat antirabbit IgG conjugated to horseradish peroxidase (HRP) was purchased from Miles Laboratories (Elkhart, Indiana). The supply of 3,3'-diaminobenzidine and bovine serum albumin (BSA) was from Sigma Chemical Co. (St. Louis, Missouri). Calf serum, Dulbec-

co's modified Eagle's medium, and Hanks' balanced salt solution (HBSS) were purchased from GIBCO (Grand Island, New York). All other reagents were purchased from common suppliers and were of reagent grade.

**Acquisition of Tissues** Normal skin specimens from sites of thick palmar skin and thin breast skin were collected from patients undergoing elective plastic surgery at Vanderbilt or Veterans Administration Medical Center in Nashville, Tennessee. Punch biopsies were obtained using 1% lidocaine anesthesia from 12 patients with psoriasis vulgaris from involved and uninvolved skin before starting UV radiation and topical therapy with corticosteroids and/or tar.

**Localization of [<sup>125</sup>I]EGF Binding by Autoradiography** Epidermal growth factor was labeled with <sup>125</sup>I by the chloramine-T method, as previously described [12], to a specific activity of  $1.5 \times 10^8$  cpm/ $\mu$ g. The [<sup>125</sup>I]EGF bound to A-431 cells and fibroblasts in a concentration-dependent, saturable, and reversible manner [13,14]. Excess unlabeled (2  $\mu$ g) EGF was added after [<sup>125</sup>I]EGF binding to show the extent of nonspecific or non-displaceable [<sup>125</sup>I]EGF.

Localization of [<sup>125</sup>I]EGF binding sites by autoradiography in sections of human skin was performed as previously described [1,2]. In brief, skin samples were sliced into very small pieces (1 mm<sup>2</sup>). Samples were incubated for 90 min at 25°C with shaking in a medium consisting of 0.5 ml HBSS containing 1 mg/ml BSA, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4 (binding buffer), and 10 ng/ml [<sup>125</sup>I]EGF. Unbound EGF was removed by repeated washes in the binding buffer at 25°C. All skin samples were fixed for 4 h in 10% neutral buffered formalin and processed for light microscopy in paraffin. Sections (6  $\mu$ m) were dipped in a 1:1 mixture of NTB-2 emulsion and distilled water. Following exposure at 4°C for 4–12 weeks, the emulsion was developed for 2 min in Kodak D-19 developer at 10°C. Sections were stained with hematoxylin and eosin and mounted in Permount. Photographs were made using an Olympus PM-10-M microscope with both bright-field and dark-field illumination.

**EGF Receptor Antiserum—Production and Characterization** The affinity-purified EGF receptor preparation, used to immunize rabbits to produce a specific antiserum to the receptor, was prepared as previously described [15,16]. Briefly, the EGF receptors from A-431 human epithelioid carcinoma membrane vesicles were solubilized and placed on an EGF affinity column. The receptor was eluted with 5 mM ethanolamine, pH 9.7, containing 10% glycerol and 0.2% Triton X-100 and then electrophoresed by the method of Laemmli [17]. The Coomassie Blue-stained receptor band was then cut out and approximately 5  $\mu$ g receptor protein injected into the rabbit in complete Freund's adjuvant. One week later, antiserum collection was begun. The IgG fraction of the antiserum was purified by ammonium sulfate precipitation [18]. The antiserum (no. 163) cross-reacted and immunoprecipitated native EGF receptors from a number of normal tissues including normal human skin [1,2] and placenta [19]. The antisera also cross-reacted with precursor and intracellular degradation forms of the EGF receptor [15,16].

**Localization of Immunoreactive EGF Receptor Molecules** Cryostat-sectioned tissue sections (4–6  $\mu$ m) were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS), pH 7.4, for 15 min at 25°C. The sections were rinsed and then incubated 15 min with 3% goat serum and 1 mg/ml glycine in PBS. Sections were treated for 4 h at 25°C with either purified normal rabbit IgG or anti-EGF receptor IgG diluted to 0.05 mg/ml in goat serum/glycine/PBS. After extensive washing, the sections were incubated for 1 h with goat antirabbit IgG conjugated to HRP diluted to 1:100 in goat serum/glycine/PBS. After rinsing, tissue sections were reacted with 0.05% diaminobenzidine and 0.01% H<sub>2</sub>O<sub>2</sub> in 0.05 Tris buffer at pH 7.6.

**[<sup>125</sup>I]EGF Binding Assay** The epidermis was separated from the dermis by incubation of 4-mm<sup>2</sup> punch biopsies in 1 M NaBr for 1 h at 37°C. The epidermis was washed once with PBS and then cut into 16 pieces. Each piece of epidermis from normal thick or involved and uninvolved psoriatic skin was additionally sliced horizontally so that the thicknesses of the pieces were similar. This tissue size (0.25 mm<sup>2</sup>) was chosen because smaller samples were too difficult to handle and larger samples bound less EGF at early times, presumably due to a slowed penetration of the ligands through the bigger samples (data not shown). A single piece of 0.25 mm<sup>2</sup> was used per assay with an interassay variation of approximately  $\pm 10\%$ . Using this sample size, it was then possible to measure total EGF binding, nonspecific binding, and to perform morphologic studies from a single biopsy. The binding mixture in a total volume of 0.1 ml contained 50 ng/ml of [<sup>125</sup>I]EGF in binding buffer at 25°C for the indicated times. Nonspecific binding was defined as [<sup>125</sup>I]EGF bound in the presence of a 100-fold excess of unlabeled EGF and generally ranged between 35–45% of the total counts bound by the 0.25-mm<sup>2</sup> samples under optimal conditions. To terminate the binding reaction, the medium was removed by aspiration and the samples washed once in ice-cold binding buffer and then washed twice with binding buffer without BSA. Samples were transferred to 12  $\times$  75-mm tubes, solubilized in 0.1 ml 1 M NaOH for 1 h at 37°C, and radioactivity measured in a  $\gamma$ -spectrometer. The protein content of each epidermal sample was determined by the method of Lowry et al [20].

**EGF-Stimulated Phosphorylation Assay** Frozen human mammary epidermis was scraped off the dermis and immediately placed in ice-cold PBS. The broken epidermis was pelleted by centrifugation at 8,000 g for 5 min. It was then homogenized in a 10  $\times$  volume ethylene glycol bis( $\beta$ -aminoethyl ether)N,N,N,N-tetraacetic acid, 3 mM iodoacetic acid in 20 mM HEPES buffer, pH 7.4, using a ground-glass homogenizer. Nuclei and unbroken cells were precipitated by centrifugation at 600 g spin. The membrane fraction of the supernatants was precipitated by centrifugation at 100,000 g for 1 h. The pellet was resuspended in 20 mM HEPES buffer, pH 7.4. A-431 cell monolayers grown as previously described [13] were washed 3 times with PBS, scraped, and homogenized in the homogenization buffer. The membrane fraction of the supernatant was precipitated and suspended as described above.

The phosphorylation assay mixture contained 75  $\mu$ g epidermal membranes or 15  $\mu$ g A-431 membranes, 0.1% Triton X-100, 2 mM MnCl<sub>2</sub>, 10  $\mu$ M vanadate, 0.25 mg/ml BSA, 1  $\mu$ Ci [ $\gamma$ -32]ATP (5 Ci/ $\mu$ mol) (ICN, Irvine, California) in 20 mM HEPES, pH 7.4, in the absence or presence of 10 ng/ $\mu$ l EGF in a total volume of 60  $\mu$ l. The reaction proceeded for 10 min at 0°C and was stopped by the addition of 60  $\mu$ l of 2  $\times$  Laemmli sample buffer [17]. After heating the samples to 95°C for 5 min, they were placed on a 6% polyacrylamide gel, electrophoresed, and the dried gel placed against a sheet of Kodak X-O Omat AR film. The film was exposed at 70°C with a Dupont Lightening Plus screen for one day.

**Protein and DNA Assays** Protein content was determined by the method of Lowry et al [20] using BSA as a standard. Optical densities were read at 500 nm on a Gilford 2600 spectrophotometer. Additional samples were used to make determinations of DNA by the method of Erwin et al [21]. For DNA analysis, a 4-mm<sup>2</sup> punch biopsy was incubated in 0.5 ml NaBr (Fisher, Springfield, New Jersey) for 1 h at 37°C. Following a rinse with PBS, the epidermis was peeled away and solubilized for 1 h at 37°C in 0.1 ml of NaOH. To the solubilized epidermis or 100- $\mu$ l aliquots of calf thymus DNA (Worthington, New Jersey), 0.5 ml of trichloroacetic acid was added and the mixture heated at 90°C for 30 min. Supernatants were cooled to 4°C and microfuged for 5 min. Supernatants were incubated at 60°C for 1 h with 100  $\mu$ l or 200 mg/ml 3,5-diaminobenzoic acid HCl (Aldrich, Milwaukee, Wisconsin). The reaction was stopped by the addition

of 2 ml of 1 N HCl. The fluorescence was measured at an excitation of 400 and an emission of 520 nm on a Turner fluorometer.

RESULTS

Autoradiography of [<sup>125</sup>I-EGF] Binding Sites

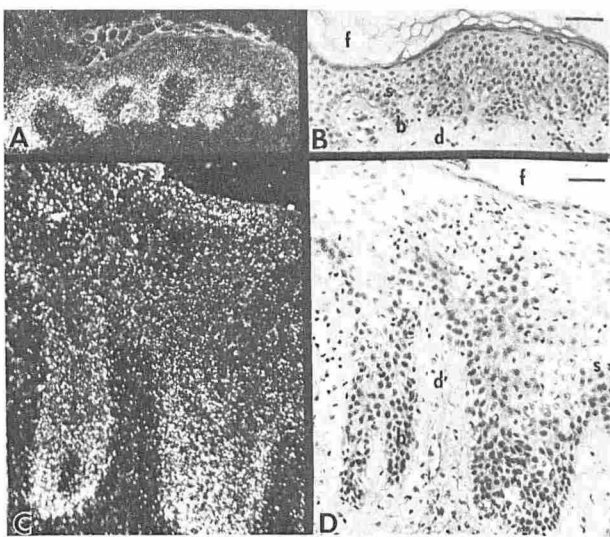
**Active Psoriatic Lesions:** We have previously shown that [<sup>125</sup>I]EGF binding sites in normal human skin were found primarily in the basal and suprabasal keratinocytes [1,2]. This EGF binding was specific, saturable, and reversible. The number of EGF receptors as measured by an ultrastructural method with an anti-EGF receptor antibody in the same skin samples correlated well with previously reported [<sup>125</sup>I]EGF binding sites [1,2] and the quantitative autoradiography (Table I).

When biopsies from psoriatic lesions were incubated in [<sup>125</sup>I]EGF, a marked increase and alteration in distribution of specific labeling was observed (Fig 1C,D, Table I). Increased [<sup>125</sup>I]EGF binding was due to the increased presence of binding sites in the upper strata of the epidermis as well as a substantial increase in epidermal thickness in psoriatic skin (Fig 1C,D) as compared with uninvolved adjacent epidermis from the same patient (Fig 1A,B) and normal skin (data not shown) [1,2]. The involved psoriatic epidermis was more cellular (Fig 1C,D) than “uninvolved” epidermis (Fig 1A,B). The number of silver grains (representing [<sup>125</sup>I]EGF binding) in psoriatic epidermis was also highest over those areas that contained the greatest density of nuclei (Fig 1D). Nuclei were present in the upper psoriatic epidermis in parakeratotic foci, and [<sup>125</sup>I]EGF binding activity was heavier in these areas. In regions where normally differentiating corneocytes (without nuclei) were present, no silver grains were present (data not shown).

Morphometric analysis of silver grains was conducted on psoriatic lesions. Although the lower epidermal compartment (defined as the first 3 basal cell layers of both psoriatic and normal skin) had a similar capacity to bind EGF, the upper compartments did not have the same EGF binding capacity (Table I). Approximately twice as many grains were detected in the upper compartments of psoriatic skin as were found in normal skin (Table I). In conclusion, EGF binding in active psoriatic lesions is unusual in that the binding sites were not primarily localized to the stratum basalis as was EGF binding in normal skin [1,2].

An apparently paradoxical result is observed when the grains per square micrometer are compared between psoriatic and thin epidermis. This is a somewhat invalid comparison since the basal layer (which binds very high levels of EGF) in thin skin represents proportionately a higher estimated percentage of the epidermis than in psoriatic skin (53% vs 25%, respectively; Table II). Thus, psoriatic skin must be compared with skin of similar thickness in this respect.

**Uninvolved Psoriatic Skin:** In 3 of the 12 patients, the skin adjacent to the active psoriatic lesions was clinically normal. However, by histologic examination, a slight increase in epidermal thickness was noted (Fig 1A,B). The pattern of [<sup>125</sup>I]EGF binding was intermediate between that of normal skin and apparently normal uninvolved skin taken from a distant site from a psoriatic patient. Therefore, the intensity of [<sup>125</sup>I]EGF binding in “uninvolved” skin varied from levels that approached those seen in the active lesions,



**Figure 1.** Autoradiographs of [<sup>125</sup>I]EGF binding in human epidermis. Punch biopsy specimens from adjacent uninvolved (A,B) or involved (C,D) skin of a psoriatic patient were incubated with [<sup>125</sup>I]EGF, sectioned, and processed for autoradiography. The emulsion was exposed for 10 weeks, the section stained with hematoxylin and eosin, and viewed by light- and dark-field microscopy as described in *Materials and Methods*. In dark-field microscopy the silver grains representing [<sup>125</sup>I]EGF binding sites are white (A,C), whereas the silver grains in bright-field are black (B,D). In involved epidermis the silver grains are seen throughout the epidermis (C), whereas in the uninvolved epidermis (A) the silver grains are heavily concentrated over the stratum basalis (b) and the lower stratum spinosum (s). The dermis (d) and skin surface (f) are visible. Size bars = 100 μm.

i.e., perilesional skin, to levels which were the same as those observed in skin samples taken from normal and nonpsoriatic patients [1,2].

**Biochemical Characterization of EGF Binding** Human epidermis was readily separated from the dermis by incubating the 4-mm<sup>2</sup> punch biopsies from normal thick and thin skin in 1 M NaBr at 37°C for 1 h. Excellent separation of the psoriatic epidermis from the dermis was also possible despite the acanthotic and papillomatous nature of many specimens (Fig 2). Under the same conditions, 1 M NaBr had no detectable effect on [<sup>125</sup>I]EGF binding to A-431 cells or isolated human keratinocyte membranes (data not shown). In agreement with the morphologic data (line 3, Table I) [<sup>125</sup>I]EGF binding capacity was highest in normal thin skin, intermediate in psoriatic skin, and lowest in normal thick skin when calculated on a milligram protein basis (Fig 3). Thus, the greater the percentage of the basal layer to whole epidermis, the greater the capacity of the whole epidermis to bind EGF.

**Table I.** Quantitative Morphometric Analysis of [<sup>125</sup>I]EGF Binding in Normal Thin, Thick, and Psoriatic Epidermis<sup>a</sup>

	Thick	Thin	Psoriatic
Upper compartment	2.9	4.1 ± 0.8	8.8 ± 0.7
Lower compartment	15.5	14.7 ± 2.8	16.5 ± 1.7
Total epidermal compartment	18.8	20.5	18.95
	n = 3	n = 15	n = 8

<sup>a</sup>Binding studies were performed as in *Materials and Methods*. Values represent the mean ± SE for specific-silver grains/μm<sup>2</sup>.

**Table II.** Comparison of [<sup>125</sup>I]EGF Binding per Protein or per DNA<sup>a</sup>

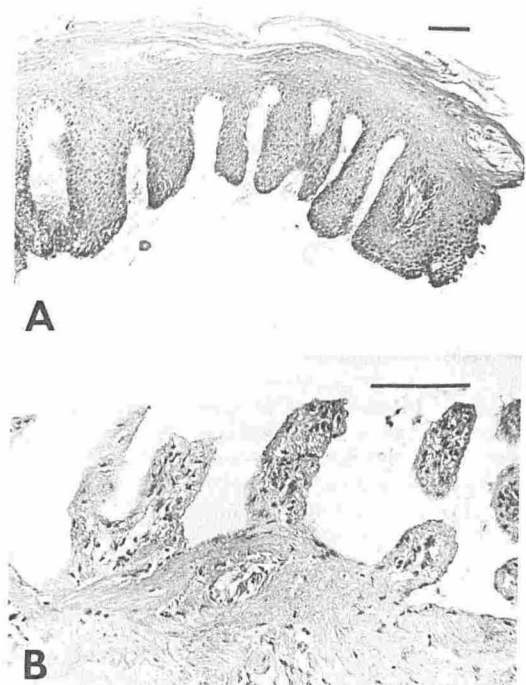
	cpm/μg Protein	Basal Protein <sup>b</sup> Total Protein	cpm/μg DNA	Basal DNA <sup>c</sup> Total DNA
Normal thin epidermis	130	0.53	12,500	0.72
Normal thick epidermis	20	0.20	5,000	0.50
Psoriatic epidermis	60	0.25	3,191	0.45

<sup>a</sup>Binding studies were performed as described in *Materials and Methods*.

<sup>b</sup>Basal protein/total protein = a rough estimate based on stratum basalis area/total area.

<sup>c</sup>Basal DNA/total DNA = a rough estimate based on the number of nucleated stratum basalis cells/total number of epidermal cells.



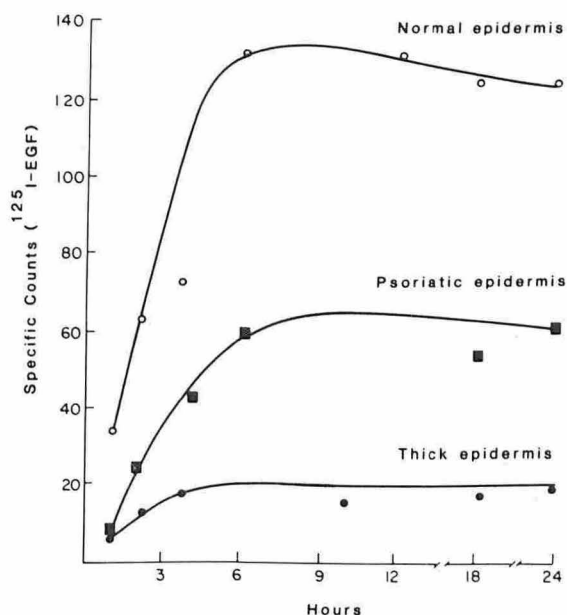


**Figure 2.** NaBr induced separation of psoriatic epidermis from dermis. Prior to [ $^{125}$ I]EGF binding studies, samples of intact normal (not shown) or psoriatic skin were incubated in 1 M NaBr for 1 h at 37°C. The epidermis (A) was peeled from the dermis (B), providing a clean separation of these skin compartments. The epidermis retained specific EGF binding sites as described in the text. H&E. Size bars = 200  $\mu$ m.

Separating the various cell layers from normal and psoriatic skin to quantify the [ $^{125}$ I]EGF binding per individual layer was technically unsatisfactory because this separation required prolonged exposure to proteases such as trypsin that affected the EGF binding (data not shown).

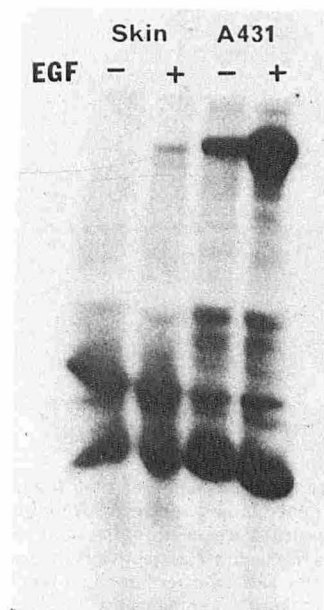
Normalization of the [ $^{125}$ I]EGF binding per unit of DNA indicated that active psoriatic lesions may actually have a decreased EGF binding capacity (Table II). These data are misleading since the proportion of nucleated or DNA-containing cells differs markedly in thin, thick, and psoriatic epidermis. Since basal keratinocytes have the greatest capacity to bind EGF (Table I) [1,2], the epidermal layers that have the greatest proportion of their nucleated cells as basal keratinocytes should have the greatest EGF binding capacity per unit of DNA, i.e., normal thin epidermis. This relationship among basal keratinocytes, nucleated cell populations, and total EGF binding per unit of DNA is indeed observed (Table II).

**EGF-Stimulated Phosphorylation in Normal Human Epidermis** To determine whether the EGF receptor in normal human skin has EGF-stimulatable autophosphorylating activity similar to that observed in other tissues, a phosphorylation assay was performed using epidermal membranes and A-431 cells. The phosphorylated membranes were solubilized, placed on a polyacrylamide gel, and electrophoresed (Fig 4). As observed in other tissues [22,23], the major phosphorylated band which was stimulated by EGF had a molecular weight of 170,000. A-431 membranes, phosphorylated under the same conditions, were included in this experiment to show that the phosphorylated band in normal human skin had the same electrophoretic mobility as the well-studied EGF receptor of 170,000 daltons in the A-431 cells [23-25]. A trace of partially degraded EGF receptor ( $M_r$  = 150,000) is also visible. Under the conditions used, EGF did not affect the phosphorylation of other membrane proteins in the extracts of normal human skin (Fig 4).



**Figure 3.** Time course of [ $^{125}$ I]EGF binding to separated epidermis from active psoriatic lesions as compared with normal thick and thin skin. Epidermis was separated by NaBr as described in Fig 2, incubated with [ $^{125}$ I]EGF in binding buffer for 1-24 h at 25°C, washed and quantitated as described in *Materials and Methods*. Under the conditions used, the peak binding activity of [ $^{125}$ I]EGF in all samples occurred at approximately 6 h.

**Immunoreactive EGF Receptors** The localization of immunoreactive EGF receptors in active and resolving psoriatic lesions is shown in Fig 5A,C. In tissue sections from active psoriatic lesions (Fig 5A), a dense immunoprecipitate was noted through-



**Figure 4.** Autoradiography of EGF-stimulated phosphorylation of human epidermal and A-431 membranes. Membranes from normal human epidermis and neoplastic A-431 cells were prepared, phosphorylated with [ $\gamma$ - $^{32}$ P]ATP in the presence or absence of EGF, and prepared for autoradiography as described in *Materials and Methods*. Under the conditions used, the EGF receptor migrates as a 170,000-dalton band whose phosphorylation is stimulated by EGF (+).

out all epidermal layers. In normal epidermis, the immunoprecipitate is found only in the stratum basalis and stratum spinosum and is absent in corneocytes (Fig 5D). In contrast, when preimmune IgG was used in place of the specific anti-EGF receptor antisera, no specific staining was observed (Fig 5B). The immunoreactive EGF receptors as localized by the immunoperoxidase method had the same qualitative distribution as the EGF receptors localized by autoradiography of [ $^{125}$ I]EGF binding sites. Thus, EGF receptors appear to be retained throughout the entire thickness of psoriatic epidermis. In contrast, immunoreactive EGF receptors are absent in the stratum corneum in regressing psoriatic lesions (undergoing successful treatment) (Fig 5C). However, receptors were still present in the stratum basalis, spinosum, and granulosum. This decrease of EGF receptor density in the more differentiated upper epidermal strata more closely resembles the pattern of immunoreactive EGF receptors that is present in normal human epidermis of comparable thickness (Fig 5D).

### DISCUSSION

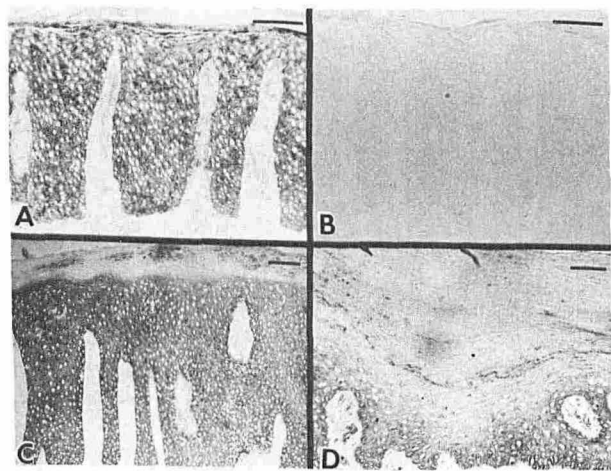
The present study indicated that the distribution of [ $^{125}$ I]EGF binding and immunoreactive EGF receptors was altered in active lesions of psoriasis vulgaris in that twice as many EGF receptors were present in the uppermost layers of skin when compared with normal skin. The distribution of EGF receptors in regressing psoriatic lesions appears to be similar to that of active psoriatic lesions, with the exception of the stratum corneum, where both EGF receptors and nuclei have begun to disappear. In the immunohistochemical studies, the anti-EGF receptor antibody used recognizes both occupied and unoccupied EGF receptors, precursor forms of the EGF receptor, and degraded forms of the EGF receptor [15,16]. Therefore, these 2 methods were useful to determine the validity of the EGF receptor localization, as each method studied different aspects of the EGF receptor under different conditions but in the same samples. Biochemical measurement of the EGF binding also showed that there was an increase in EGF receptors in psoriatic skin as compared with

normal skin of comparable thickness. Persistence of [ $^{125}$ I]EGF binding and immunoreactive EGF receptors in the active lesions of psoriasis vulgaris suggests that EGF may have a role in epidermal homeostasis. The decrease or return toward normal levels and distribution of EGF receptors in regressing lesions of psoriasis vulgaris could have important implications in the clinical management of this disease.

In our previous studies on normal human epidermis [1,2], [ $^{125}$ I]EGF binding or immunoreactive EGF receptors primarily occurred in the first 2 or 3 layers of basal and suprabasal keratinocytes. In the present study, the level of EGF receptors detected in the active lesions of psoriasis was also highest in the lower compartment and did not significantly differ in number of EGF receptors. Thus, our data do not support the hypothesis that an increase in EGF receptors should occur only on the mitotically active cells in psoriasis. These data are in accord with previous studies showing that, even in psoriasis, the dividing cells were found primarily in the first 3 layers of basal and suprabasal keratinocytes [6-9]. How these findings relate to the abnormal proliferation in psoriasis and abnormalities in other hormonal and enzymatic differences in psoriatic skin such as cyclic nucleotides [26], phospholipase A<sub>2</sub> [27], calmodulin [28,29], or plasma membrane glycoproteins [30-32] is unknown. Our preliminary studies with other hyperproliferative skin diseases (lichen planus, verruca vulgaris, condyloma acuminatum, basal cell and squamous cell carcinoma) have not produced the same distribution of EGF receptors seen in psoriasis vulgaris.

Whether these [ $^{125}$ I]EGF binding sites and immunoreactive EGF receptors detected in the involved psoriatic skin in the present study represent biologically active EGF receptor with its associated tyrosyl kinase activity is also not known. Epidermal growth factor stimulated the phosphorylation of a 170,000 dalton protein in extracts of normal human epidermis (Fig 4), presumptively the EGF receptor with its intrinsic tyrosine kinase activity. However, Gentleman et al [33] recently showed that tyrosyl kinase and phosphotyrosyl phosphatase activity were increased 2-fold in active lesions of psoriasis compared with uninvolved skin. In their studies the tyrosyl kinase activity in these studies was not stimulated by EGF or platelet-derived growth factor (PDGF) when the samples were solubilized. Whether this increased tyrosyl kinase and phosphatase activity was actually due to EGF, another known growth factor such as PDGF, a virus or oncogene tyrosyl kinase was not answered. The amount of tissue required to prove that the EGF receptor kinase activity is increased 2-fold prevented us from resolving this question. Morphometric analysis of silver grains in the present study independently indicates a 2-fold increase in [ $^{125}$ I]EGF binding in the upper layer of psoriatic epidermis so that the most likely source of the increased tyrosyl kinase activity in psoriatic lesions is that of the EGF receptor.

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**Figure 5.** Immunoperoxidase staining of EGF receptors in normal and psoriatic epidermis. Punch biopsy specimens from involved psoriatic lesions (A,B), treated psoriatic lesions from the same patient (C), and normal thick palmar skin (D) were sectioned, washed, incubated with normal IgG (B) or with specific anti-EGF receptor IgG sera (A,C,D), and processed for immunoperoxidase staining as previously described [2] and in *Materials and Methods*. In active psoriatic lesions (A), immunoreactive EGF receptors were present in all epidermal layers, whereas no specific staining was seen in the preimmune control sections (B). When the lesion began to regress while the patient was receiving UV therapy, immunoreactive EGF receptors were markedly reduced in the stratum corneum (C). In comparably thick normal human skin, immunoreactive EGF receptors were primarily present in the stratum basalis and nearly absent from the stratum granulosum and stratum corneum. Size bars = 100  $\mu$ m.

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